

Characterization of microsphere and binding performance

DNA loaded microspheres exhibited polymorphic colloid shape with a polydispersed particle size of less than 3 microns as determined by light microscopy. Purified microspheres were stable for at least one month without appreciable degradation. The loading level for LAMP-1 plasmid DNA was 20% (w/w). The encapsulation efficiency was typically >95%. The mobility of the free LAMP-1 DNA and the released DNA (from the microsphere) in 1% agarose gel electrophoresis were identical (FIG. 2), suggesting that the encapsulated DNA was released in its original form. Release rate of the cDNA from the microspheres was dependent on the crosslinking density and on the enzyme level (FIG. 4). Sustained release of up to weeks can be readily obtained.

We tested the ability of the LAMP-1 DNA loaded microspheres to bind and subsequently transfect a human histiocytic lymphoma cell line (U937) in tissue culture. When coated with either anti-LFA or anti-CD44 monoclonal antibody (both protein targets were expressed in high amount of U937 cell surface), expression LAMP-1 protein was detected by day 3 (FIG. 4A, fluorescent granules) when stained with antibodies recognizing LAMP-1. The staining pattern of U937 cells incubated with LAMP-1 microspheres was identical to the calcium phosphate method of transfection (FIG. 4B). Microspheres that were either coated with avidin or non-specific CHA mAb showed no granular staining patterns, and were identical to untreated cells (FIG. 4C).

Using flow cytometry, we showed that the expression of LAMP-1 was detected in up to 5% of U937 cells in culture (FIG. 5). None of the controls—blank microspheres, microspheres with cDNA but no antibodies, microspheres with cDNA and coated with a mismatched anti-P-glycoprotein antibody (MRK-Msp.), and free cDNA at a concentration six time higher than entrapped in the microspheres—showed any evidence of transfection. The efficiency of transfection appears to be dose-responsive. In general, the transfection efficiency of this particular gene and cell type is comparable between the proposed microspheric delivery system (1–10%) and the calcium phosphate precipitation method (2–15%). FIG. 6 demonstrates the concept in a different cell type using a different monoclonal antibody. Again, free cDNA could not transfect the cells. Eventually the LAMP-1 expression disappeared after several passages. Positive results were also obtained for the luciferase reporter gene system. Transfection was clearly detected by measurement of luciferase enzymatic activity, in 293s cells incubated with luciferase gene-loaded microspheres.

We claim:

1. A solid microparticle for delivery of nucleic acids to and transfection of target cells comprising gelatin and nucleic acids, wherein a molecular species is attached to the surface of said microparticle, wherein the molecular species is selected from the group consisting of a targeting ligand and a linking molecule, wherein the linking molecule is selected from the group consisting of avidin, biotin, and staphylococcal protein A, and further wherein the microparticle is solid and is less than 3 μ m.

2. The microparticle of claim 1 wherein the linking molecule is attached to said microparticle.

3. The microparticle of claim 2 wherein the linking molecule is covalently attached to said microparticle by means of glutaraldehyde cross-linking.

4. The microparticle of claim 2 wherein said linking molecule is avidin.

5. The microparticle of claim 4 wherein a biotinylated targeting ligand is coupled to the linking molecule.

6. The microparticle of claim 5 wherein the biotinylated targeting ligand is a biotinylated antibody.

7. The microparticle of claim 6 wherein biotin is bound to said antibody at oligosaccharide groups on the F_c portion of said antibody.

8. The microparticle of claim 2 wherein a targeting ligand is coupled to the linking molecule.

9. The microparticle of claim 8 wherein said targeting ligand is selected from the group consisting of hormones, antibodies, cell-adhesion molecules, saccharides, and neurotransmitters.

10. The microparticle of claim 1 wherein the targeting ligand is attached to said microparticle.

11. The microparticle of claim 10 wherein said targeting ligand is selected from the group consisting of hormones, antibodies, cell-adhesion molecules, saccharides, and neurotransmitters.

12. The microparticle of claim 1 wherein said microparticle comprises from 5% to 30% (w/w) nucleic acids.

13. The microparticle of claim 1 wherein said microparticle comprises 20% to 30% (w/w) nucleic acids.

14. The microparticle of claim 1 wherein said nucleic acids comprises a gene larger than 10 kb.

15. The microparticle of claim 1 further comprising chondroitin sulfate.

16. The microparticle of claim 1 wherein said nucleic acids comprise a gene of 2 to 10 kb.

17. A method of forming solid microparticles for gene delivery and transfection of target cells, comprising the steps of:

forming solid microparticles by coacervation of nucleic acids and gelatin, wherein the gelatin is at a concentration between 2% and 7%; and,

adhering a molecular species to the surface of the microparticles wherein the molecular species is selected from the group consisting of a targeting ligand and a linking molecule, wherein the linking molecule is selected from the group consisting of avidin, biotin, and staphylococcal protein A.

18. The method of claim 17 further comprising the step of: cross-linking the molecular species to the microparticles.

19. The method of claim 17 wherein a linking molecule is adhered, said method further comprising the step of:

binding a targeting ligand to the linking molecule.

20. The method of claim 19 wherein the targeting ligand is selected from the group consisting of antibodies, hormones, cell-adhesion molecules, saccharides, and neurotransmitters.

21. The method of claim 19 wherein the targeting ligand is chemically modified with a moiety which binds to the linking molecule.

22. The method of claim 17 wherein the coacervation is performed in the presence of sodium sulfate.

23. The method of claim 22 wherein the concentration of sodium sulfate is between about 7 and 43 mM in the step of coacervation.

24. The method of claim 17 wherein a targeting ligand is adhered to the surface of said microparticle, said targeting ligand being selected from the group consisting of antibodies, hormones, cell-adhesion molecules, saccharides, and neurotransmitters.

25. The method of claim 17 wherein the linking molecule is avidin.

26. The method of claim 17 wherein the nucleic acids are present in a concentration of 1 ng/ml to 500 μ g/ml in the step of coacervation.

27. A method for introducing nucleic acids into cells, in vitro, comprising the steps of:

incubating (a) cells to be transfected with (b) solid microparticles of less than 3 μ m, said microparticles com-